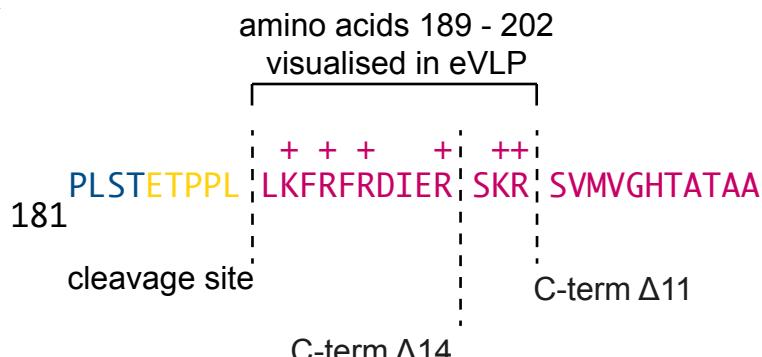
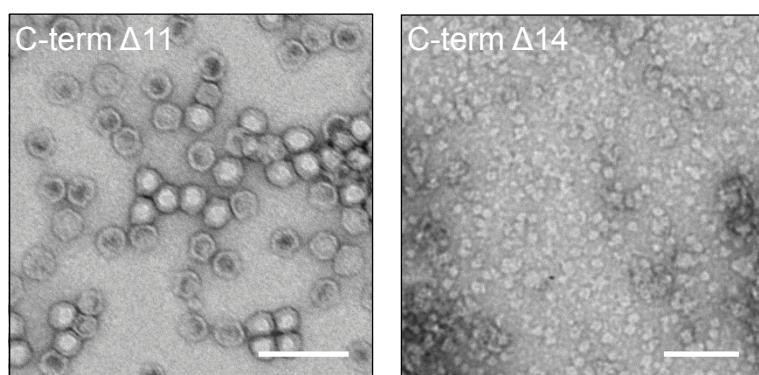


SUPPLEMENTARY FIGURES

a



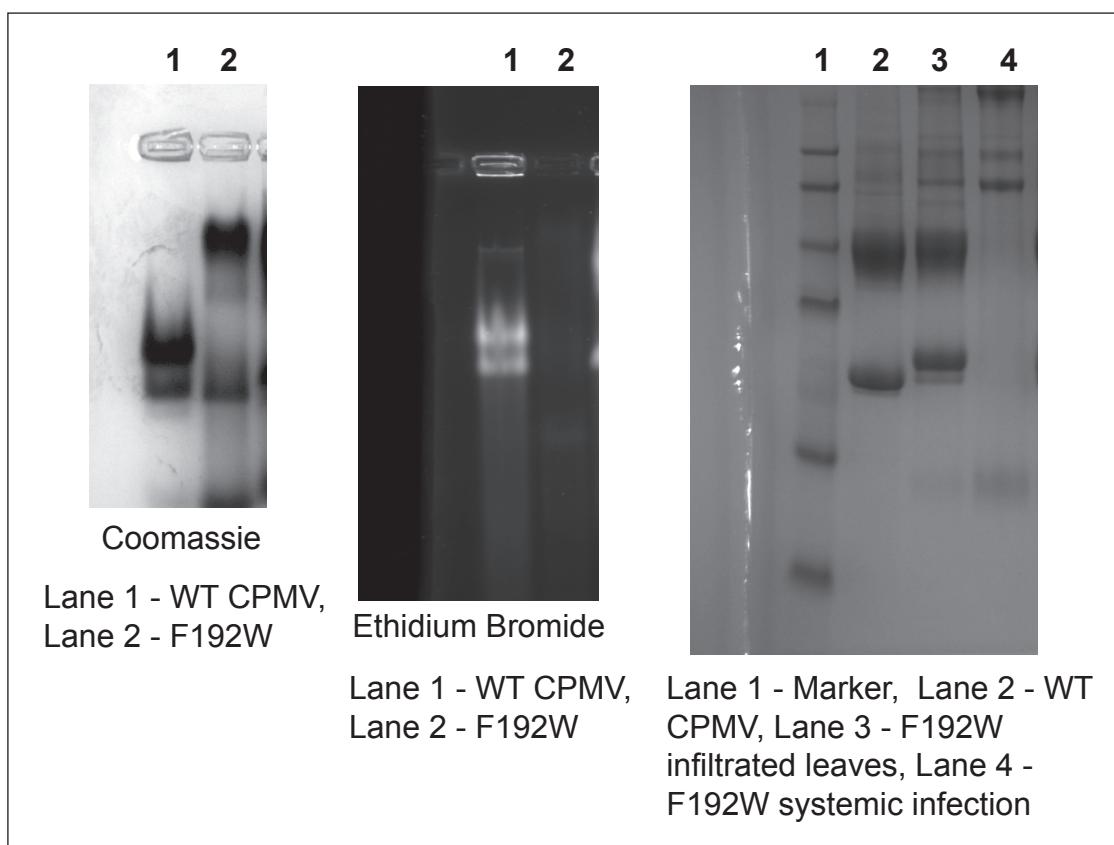
b



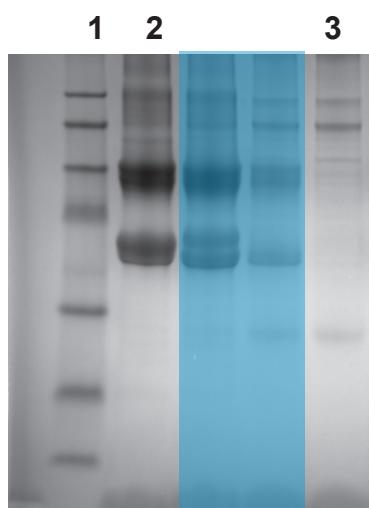
Supplementary Figure 1. CPMV eVLP C-terminal deletion mutants

- a.** The sequence of S subunit C-terminal amino acids 180 - 213. The C-terminal 24 amino acid segment of S subunit is cleaved following assembly, and is coloured magenta. The natural cleavage site between Leu189 and Leu190 is shown. This region of the polypeptide is highly positively charged and the positive amino acids are indicated. The positions of the C-terminal deletion mutants are also indicated. The C-term Δ11 mutant is missing the C-terminal 11 amino acids and the C-term Δ14 mutant is missing the C-terminal 14 amino acids.
- b.** Negative stain electron microscopy illustrating the C-term Δ11 mutant can form particles, however the C-term Δ14 mutant does not form particles. Scale bars are 100 nm.

Gels from figure 5a

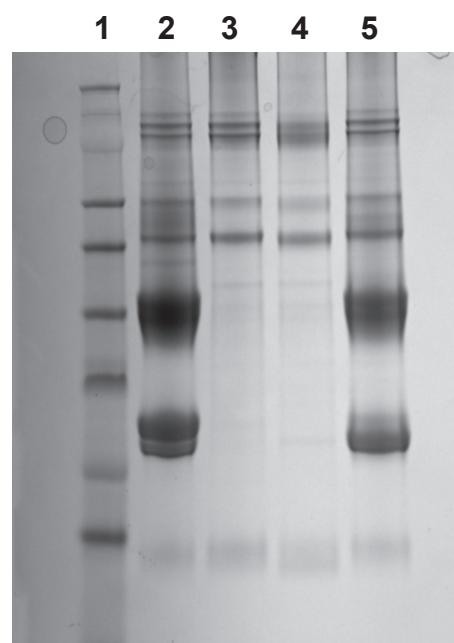


Gels from figure 5b



Lane 1 - Marker, Lane 2 -
WT eVLP, Lane 3 - V109W

Gel from figure 5c

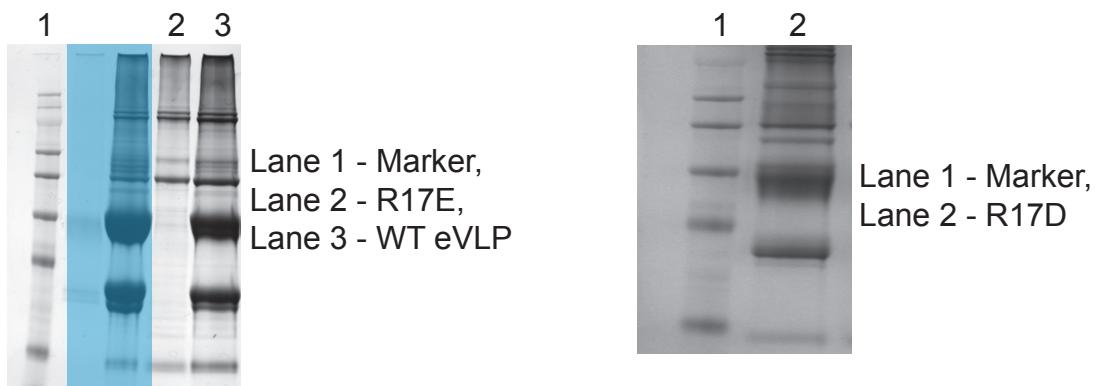


Lane 1 - Marker, Lane 2 - WT eVLP,
Lane 3 - R193D, Lane 4 - E147R,
Lane 5 - E147R/R193D

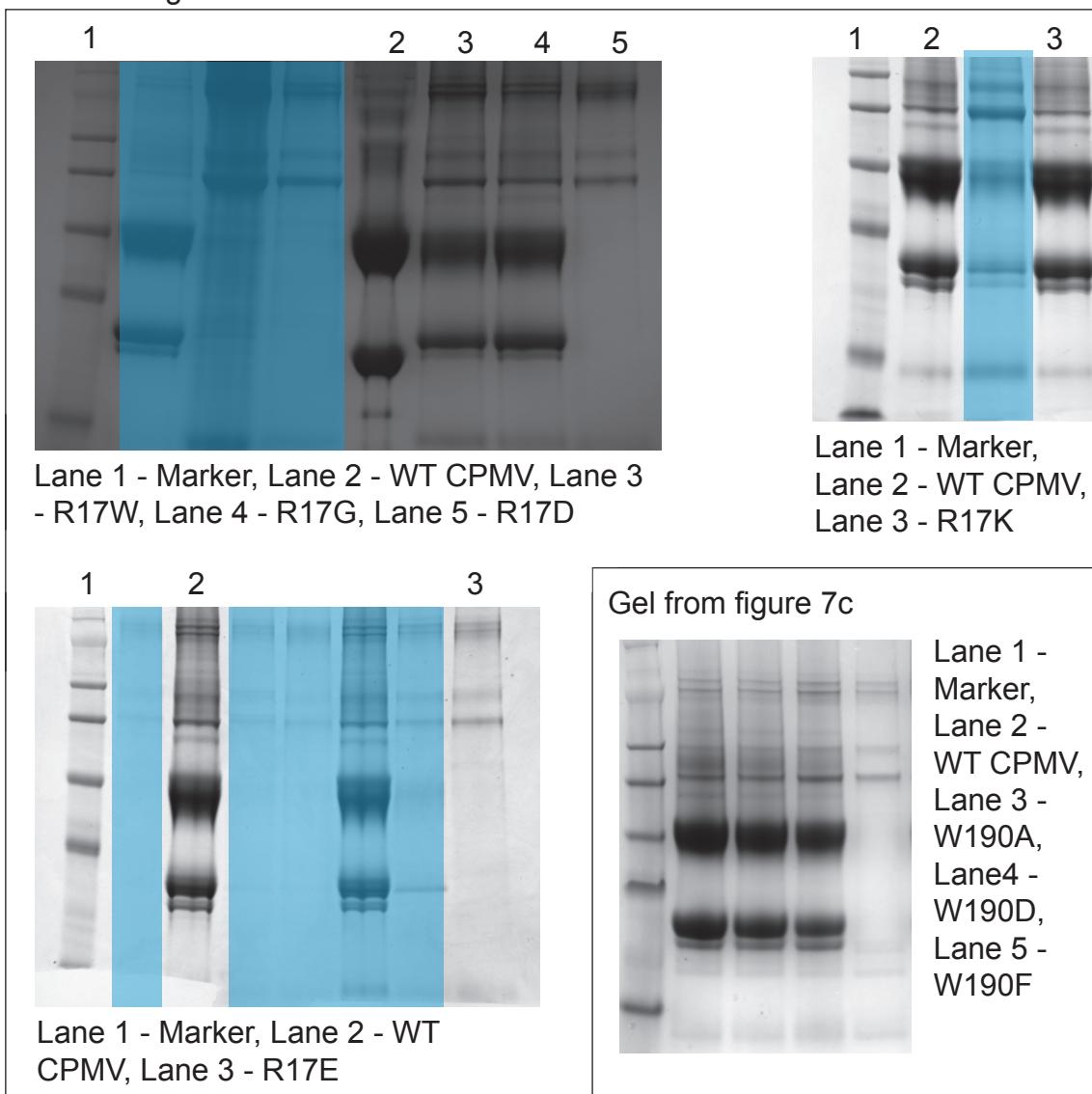
Supplementary Figure 2 - Gels from figure 5

Uncropped images of the lanes analysed to provide data for this manuscript in figure 5. Lanes not described are covered in a transparent blue box to avoid confusion.

Gels from figure 7a



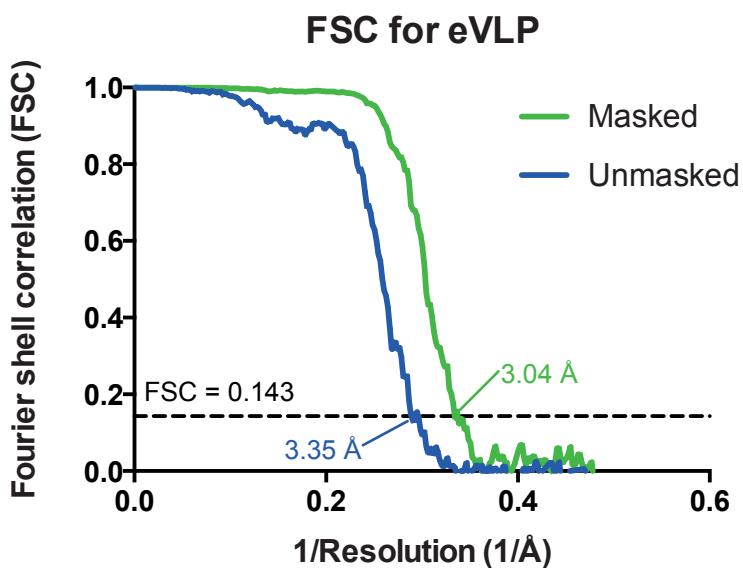
Gels from figure 7b



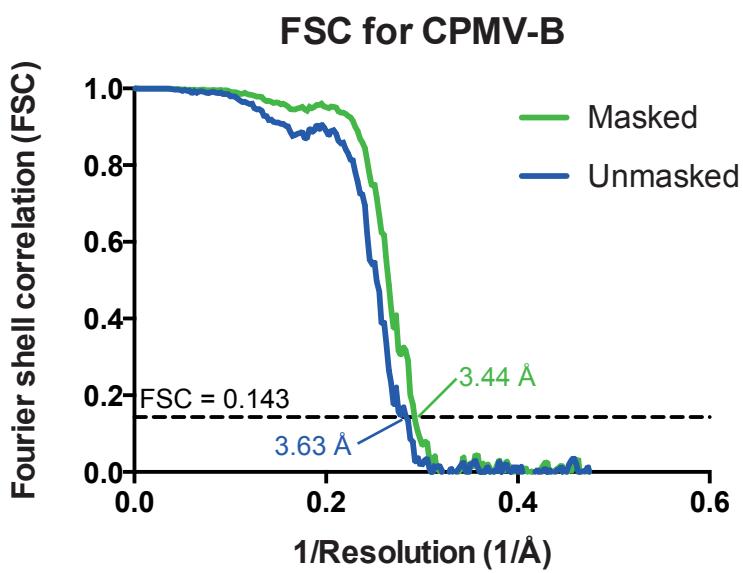
Supplementary Figure 3 - Gels from figure 7

Uncropped images of the lanes analysed to provide data for this manuscript in figure 7. Lanes not described are covered with a transparent blue box to avoid confusion.

a



b



Supplementary Figure 4. Fourier shell correlation plots for eVLP and CPMV-B

Gold-standard Fourier shell correlation (FSC) plot for CPMV eVLP (a) and CPMV-B (b). The FSC plots were calculated for the final masked map (green) and the unmasked map (blue). Reported resolutions were based on $\text{FSC} = 0.143$ criteria.

SUPPLEMENTARY TABLES

Supplementary Table 1. C-terminal deletion mutants for eVLP CPMV

Deletion mutant	Number of C-terminal amino acids cleaved	Yield	Assembly
C-term Δ7	7	Wild-type (WT)	+++
C-term Δ11	11	WT	+++
C-term Δ14	14	Very low)	+
C-term Δ16	16	Very low	+
C-term Δ24	24	Very low	+

Supplementary Table 2. Effect of mutations on eVLPs assembly and yield after transient expression.

Mutation	Capsid assembly	Yield
Wild-type (WT)	Yes	as WT
F192W (S)	Yes	WT
F192H (S)	Yes	WT
F192Y (S)	Yes	WT
F194W (S)	Yes	WT
V109W (S)	No	No
V109D (S)	No	No
R193G (S)	No	No
R193D (S)	No	No
E147R (S)	No	No
E147R/R193D (S)	Yes	WT
R195G (S)	Yes	WT
R17D (L)	Yes	WT
R17E (L)	No	No

CPMV eVLP mutants were designed and transiently expressed (as described in the methods). The ability of the eVLP to form particles and the yield produced were analysed by SDS-PAGE and negative stain electron microscopy (EM). Mutations of small (S) subunit and large (L) subunit are indicated in brackets.

Supplementary table 3. Effect of mutations on CPMV capsid assembly, RNA encapsidation and systemic transport during infection.

Mutation	Capsid assembly	RNA packaging	Systemic transport
F192W (S)	Yes	No	No
F192H (S)	Yes	Yes	Yes
F192Y (S)	Yes	Yes	Yes
F194W (S)	Yes	Yes	No
R17D (L)	Low	No	No
R17E (L)	No	No	No
R17W (L)	Yes	Yes	Yes
R17K (L)	Yes	Yes	Yes
R17G (L)	Yes	Yes	Yes
W190A (L)	Yes	Yes	Yes
W190D (L)	Yes	Yes	Yes
W190F (L)	No	No	No

WT CPMV genomic RNA was mutated and CPMV particles produced as described in the methods. The ability of the CPMV mutants to form particles, the yield produced and the ability to produce systemic infection were analysed by SDS-PAGE and negative stain EM. Mutations of small (S) subunit and (L) large subunit are indicated in brackets.

Supplementary Table 4. Primers used for site-directed mutagenesis

Primer name	Primer sequence (5' – 3')
R17K-F	CCTTCCTTGGATGATACAGGCTCAGTTAAGGGTTCTTGCTTGACACAAAATTG
R17K-R	CGAATTTGTGTCAAGCAAAGAACCCCTAACTGAGCTGTATCATCCAAAGAAAGG
R17E-F	CTTCTTGGATGATACAAGCTCAGTTGAGGGTTCTTGCCTTGACACAAAATTG
R17E-R	GAATTTGTGTCAAGCAAAGAACCCCTCAACTGAGCTGTATCATCCAAAGAAAG
R17A-F	TTTGGATGATACAAGCTCAGTTGCTGGTTCTTGCTTGA CAC
R17A-R	GTGTCAAGCAAAGAACCCAGCAACTGAGCTGTATCATC CAAA
R193G-F	ACGGAAAACCTCCACCGTTATTAAAGTTGGGTTCGGG ATATT
R193G-R	AATATCCGAAACCCAAACTTAATAACGGTGGAGTTTC CGT
E147D-F	ATCAGACCACCTGGTATCTGATTGTGTTGCTACC
E147D-R	GGTAGCACACAATCAAGAATACCAGGTGGTTCTGAT
E147R-F	ATCAGACCACCTGGTATCTGACTGTGTTGCTACC
E147R-R	GGTAGCACACAGTCAAGATACCAGGTGGTCTGA
F192W-F	GTCAACGGAAACTCCACCGTTATTAAAGTGGAGGTTTC GGGATATT
F192W-R	AATATCCGAAACCTCCACTTAATAACGGTGGAGTTTC CGTTGAC
F192Y-F	GGAAACTCCACCGTTATTAAAGTATAGGTTCGGGATAT

	TGA
F192Y-R	TCAATATCCGAAACCTATACTTTAATAACGGTGGAGTT TCC
F192H-F	CAACGGAAACTCCACC GTTATTAAAGCATAGGTT CGG GATATTGAAC
F192-R	GTTCAATATCCGAAACCTATGCTTAATAACGGTGGAG TTTCCGTTG
F194W-F	GGAAACTCCACC GTTATTAAAGTTAGGTGGCGGGATA TTGAACGC
F194W-R	GCGTTCAATATCCGCCACCTAAACTTAATAACGGTGG AGTTTCC
R195G-F	TCCACCGTTATTAAAGTTAGGTGGGGATATTGAACG CT
R195G-R	AGCGTTCAATATCCCCAACCTAAACTTAATAACGGTGG GA
R193D-F	CTGTCAACGGAAACTCCACCGTTATTAAAGTTGATTTT CGGGATATTGAACGC
R193D-R	GCGTTCAATATCCGAAAATCAAACCTTAATAACGGTGG AGTTCCGTTGACAG
V109W-F	GTTATGATGCGCGGACATTTGGATCTCACAA CCTGGTT CT
V109W-R	AGAACCCAGGTTGTGAGATCCAAAATGTCCCGCGCATCA TAAC
V109D-F	TATGATGCGCGGACATTTGATATCTCACAA CCTGGTTCT G
V109D-R	CAGAACCCAGGTTGTGAGATACAATGTCCCGCGCATCAT A
V42D-F	CAACGGAAAATAACTCCTGATGGTGATGACAATTGGA ATA

V42D-R	TATTCCAATTGTCATCACCATCAGGAGTTATTTGCCGT TG
V42W-F	GACTTAATCAACGGAAAATAACTCCCCGGGGTGTGA CAATTGGAATA
V42W-R	GTGCGTATTCCAATTGTCATCACCCCAAGGAGTTATTT GCCGTTGATTAAGTC
W190A-F	CACCATTACCACTTGGCTGATTGTCAGAATGCTTACC CCTTAATCGTTG
W190A-R	CAACGATTAAGGGTAAAGCATTCTGACAATCAGCAA GTGGTAAATGGTG
W190F-F	CCACTTGGCTGATTGTCAGAATTTTACCCCTTAATCG TTGGA
W190F-R	TCCAACGATTAAGGGTAAAAATTCTGACAATCAGCAA GTGG

F – forward primer

R - reverse primer

Supplementary Table 5. Primers used for making deletion mutants in the C-terminus of the small coat protein

Primer name	Primer sequence (5' – 3')
DM-F	TACAAT <u>CTCCGGAAG</u> ATTTAATCTTGG
DM24-R	GAC <u>AGGCCT</u> TATAACGGTGGAGTTTC
DM17-R	TAC <u>AGGCCT</u> CTAAATTATCCGAAACCT
DM7-R	TAC <u>AGGCCT</u> CTAACCTAAACACTACG
DM11-R	TAC <u>AGGCCT</u> CTAACGCTTGGAGCGTTCAATATCCCG

Underline indicates position of restriction site, either BspEI or StuI.